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# Human Colon Microbiota Transform Polycyclic Aromatic Hydrocarbons To Estrogenic Metabolites

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Estrogenic PAH metabolites from colon microbiota

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**Abbreviations:**

PAH: polycyclic aromatic hydrocarbon

EE2: 17α ethynyl estradiol

LC-MS: liquid chromatography – mass spectrometry

Ah: Aryl hydrocarbon

GI: gastrointestinal

DW: dry weight

GC-MS: gas chromatography – mass spectrometry

SHIME: simulator of the human intestinal microbial ecosystem

**Abstract.**

Ingestion is an important exposure route for polycyclic aromatic hydrocarbons (PAH) to enter the human body. Although the formation of hazardous PAH metabolites by human biotransformation enzymes is well documented, nothing is known about the PAH transformation potency of human intestinal microbiota. Using a gastrointestinal simulator, we show that human intestinal microbiota can also bioactivate PAHs, more in particular to estrogenic metabolites. PAH compounds are not estrogenic and indeed, stomach and small intestine digestions of 62.5 nmol naphthalene, phenanthrene, pyrene and benzo(a)pyrene showed no estrogenic effects in the human estrogen receptor bioassay. In contrast, colon digests of these PAH compounds displayed estrogenicity, equivalent to 0.31, 2.14, 2.70 and 1.48 nmol 17α ethynyl estradiol (EE2), respectively. Inactivating the colon microbiota eliminated these estrogenic effects. Liquid chromatography – mass spectrometry (LC-MS) analysis confirmed the microbial PAH transformation by the detection of PAH metabolites 1-hydroxypyrene and 7-hydroxybenzo(a)pyrene in colon digests of pyrene and benzo(a)pyrene. Furthermore, we show that colon digests of a PAH contaminated soil (simulated ingestion dose of 5 g $d^{-1}$) displayed estrogenic activity equivalent to 0.58 nmol EE2, whereas stomach or small intestine digests did not. Although the matrix in which PAHs are ingested may lower the exposure concentrations in the gut, our results imply that the PAH bioactivation potency of colon microbiota is not eliminated by the presence of soil. Moreover, since PAH toxicity is also linked to their estrogenicity, the PAH bioactivation potency of colon microbiota suggests that current risk assessment may underestimate the risk from ingested PAHs.

**Introduction.**

Polycyclic aromatic hydrocarbons (PAH) are high priority environmental contaminants because of their toxic, carcinogenic and putative estrogenic or anti-estrogenic properties in the human body. Human exposure to high molecular weight PAHs mainly occurs through oral uptake of charcoil-broiled, grilled and smoked meats (van Maanen et al. 1994) and through ingestion of soil or poorly cleaned vegetables, resulting in exposed doses about an order of magnitude higher than exposure by inhalation (Heisterkamp and van Veen 1997). The hazardous effects of ingested PAHs come from this PAH fraction that releases from the nutrition, soil or associated organic matter in the intestinal lumen and which, upon intestinal absorption, reaches the intestine enterocytes and liver hepatocytes. In these cells, PAHs may act as ligands to the human aryl hydrocarbon (Ah) receptor, which plays a central role in the toxic response of specific aromatic hydrocarbons by the regulation of typical human biotransformation enzymes (reviewed by Hankinson 1995).

The risk from orally ingested PAHs is currently thought to be reduced when co-ingested soil or fibres decrease the intestinal PAH absorption and hence, bioavailability (De Kok and van Maanen 2000). The majority of ingested PAHs would pass harmlessly through the gastrointestinal (GI) tract without being transformed by human enzymes to hazardous metabolites. However, this assumes that no microbial biotransformation of PAHs occurs. The human GI tract harbors an incredibly diverse microbial community, which typically performs fermentative processes, but which is also capable of transforming xenobiotic compounds (Aura et al. 2002; Ilett et al. 1990; Macdonald et al. 1983). Hence, if microbial PAH biotransformation in the human colon is possible, the susceptibility of the colon epithelium to bioactive PAH metabolites may increase the health risks that are associated with non-absorbed PAHs that reach the colon. To date, no information is available on the PAH bioactivation potency from human colon microbiota. To evaluate this, we looked in this study

at PAH estrogenicity, since several PAH metabolites structurally resemble steroidal hormones that bind the human estrogen receptor (Ariese et al. 2001), which could thus lead to estrogenic or anti-estrogenic activity *in vivo.*

We opted for an *in vitro* approach to specifically look for microbial biotransformations and thus avoid possible interference from colon epithelium enzymes that would be present in an *in vivo* approach. Pure PAH compounds and a PAH contaminated urban soil were incubated in the stomach, small intestine and colon suspensions from a simulator of the human GI tract. Given the aromaticity of PAHs, we used a modified aryl hydrocarbon (Ah) receptor yeast assay (Miller 1997) to investigate whether the PAHs in the different digests could activate the human Ah receptor and subsequently induce signal transduction. Besides this, we investigated the estrogenicity of the PAH incubated digests by monitoring activation of the human estrogen receptor in a modified estrogen receptor yeast assay (Routledge and Sumpter 1996). In addition, we applied a newly optimized liquid chromatography – mass spectrometry (LC-MS) protocol to detect whether PAH metabolites were formed during incubation.

## Materials and Methods

**Chemicals.** PAH parent compounds napthalene, phenanthrene, pyrene and benzo(a)pyrene were reagent grade and obtained from Sigma-Aldrich (Bornem, Belgium). To avoid solubility problems in the incubation tests, PAHs were first dissolved in ethanol prior to digestion. All stock solutions were prepared in amber glass bottles and stored in the dark at 4 °C. Hydroxy-PAH metabolites 1-OH naphthalene, 9-OH phenanthrene, 1-OH pyrene and 7-OH benzo(a)pyrene were reagent grade and also obtained from Sigma-Aldrich.

**Incubations.** Incubations of PAHs and soils were performed in batch by sampling gastrointestinal suspension from the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). This dynamic model of the gastrointestinal tract consists of 5 compartments representing the stomach, small intestine, colon ascendens, transversum and descendens, respectively (Figure 1). The colon suspension contains *in vitro* cultured microbiota which were isolated from human feces and which are representative for the *in vivo* colon microbial ecology after a growth stabilization period in the different colon compartments (Molly et al. 1993). A typical stomach digestion consists of an incubation of PAHs or PAH contaminated soil samples for 3 hours at pH 1.5 at 37°C. A small intestine digestion consists of an incubation for 5 hours at pH 7 at 37°C in the presence of bile salts (0.2 mmol $L^{-1}$) and pancreatic enzymes supplemented as pancreatic powder from porcine origin (0.4 g $L^{-1}$). A colon digestion consists of an incubation with colon microbiota for 48 h at 37°C, withdrawn from the colon vessels of the SHIME reactor. Some samples were incubated with inactive colon microbiota. For this, colon microbiota were autoclaved for 30 minutes (121°C, 1 bar overpressure). Incubation of PAH standard compounds in stomach, small intestine and colon digests occurred at a concentration of 20 µmol $L^{-1}$. This concentration is normally not encountered in the gastrointestinal tract, but gave us more possibilities to study microbial

PAH metabolism in depth. Gastrointestinal digestion experiments on soil samples were done as previously described (Van de Wiele et al., 2004) to simulate a hypothetical soil ingestion of 5 g $d^{-1}$ by *pica* afflicted children (stomach 40 mL, small intestine 60 mL and colon 100 mL). To avoid photocatalytic effects, all digestions were performed in amber flasks. After the respective incubations, samples were centrifuged at 3000*g* for a duration of 10 minutes to remove most of the particulates and biomass. The supernatants were subsequently stored at -20°C prior to analysis.

**Sample treatment.** PAH parent components and PAH metabolites were extracted from the digests by performing a liquid/liquid extraction in which the digest and ethyl acetate were mixed in a 1:1 ratio. The ethyl acetate fraction was subsequently put in a rotavapor to remove most of the solvent. The remainder of the solvent was removed under a gentle stream of nitrogen gas and finally replaced by dimethylsulfoxide which is a suitable solvent to use in bioassay tests. For chemical analysis of the samples using LC-MS, sample aliquots were subjected to a solid phase extraction using PrepSep$^{TM}$ C18 (250 mg) (Fisher Scientific, Edmonton, Canada). PAH hydroxylates were eluted with methanol.

**PAH conjugate analysis.** To check whether conjugated PAH metabolites were formed in the different digests, samples were also incubated in the presence of β-glucuronidase and aryl sulfatase, both obtained from Sigma–Adrich (Belgium). After the PAH parent compounds had been incubated in SHIME suspension, a 1 mL aliquot of these samples were diluted in 1mL 0.1M acetate buffer and the pH was adjusted to 5 with sodium hydroxide. A volume of 400µl β-glucuronidase (100U $mL^{-1}$) and 250µl aryl sulfatase (60U $mL^{-1}$) were added and the mixture was incubated for 6 h at 37 ◦C to hydrolyze the PAH conjugates.

**Bioassays.** For the bioassays, we used a modified protocol from De Boever et al. (2001) that was based on the protocol developed by Routledge and Sumpter (1996) for the yeast estrogen bioassay and Miller (1997) for the yeast Ah bioassay. Details on the yeast Ah bioassay, the yeast estrogen bioassay and the preparation of the medium compounds have been described before (Miller 1997; Routledge and Sumpter 1996). In brief, these researchers transformed *Saccharomyces cerevisiae* with the human Ah receptor gene and the human estrogen receptor (ERα) gene, together with expression plasmids containing responsive elements and the *lacZ* reporter gene (encoding the enzyme β-galactosidase). The expression of β-galactosidase is triggered by test chemicals, which upon binding to the respective receptors induce the conformational change necessary for binding of the receptor/ligand dimer to the responsive elements. This β-galactosidase activity is quantified at 540 nm by the conversion of the chromogenic substance chlorophenol red-β-D-galactopyranoside into chlorophenol red. The bioassay response is expressed as the absorbance at 540 nm divided by the optical density at 630 nm $(A540/A\ 630)_{net}$. Positive signals in the Ah receptor assay were typically expressed as percentage equivalence to 200 nM benzo(a)pyrene which arbitrarily corresponded to a bioassay response of 100 %. Similarly, estrogenic activity of the samples was expressed as percentage equivalence to 6.96 nM 17α-ethynyl estradiol which elicited a 100 % response in the estrogen receptor bioassay (De Boever et al. 2001). To make sure that background signals from gastrointestinal suspensions of soil or food matrices did not interfere with the detection of estrogenic signals in the bioassays, corrections were made in a set of negative control experiments by subtracting the response of a PAH containing digest with that from a blank digest without PAHs (see supplemental material). The bioassays were performed in 96-well plates in which 10μL of the test compounds were tested and incubated with 240μL of the genetically modified yeast (optical density of 0.25 at 610nm). Serial dilutions of the test

compounds were made in dimethylsulfoxide which allowed to generate dose-response curves for doses (ordinate) versus activity (abscissa). The data were fitted by a 4 parametric logistic model using the Marquardt-Levenberg algorithm (Sigmaplot 4.0, SPSS Inc., Chicago, Illinois, USA) (De Boever et al. 2001).

**PAH analysis.** Sample treatment for and determination of PAHs were performed by the Environmental Research Centre (Erembodegem, Belgium). Briefly, PAHs from pellets were extracted by a 1:1 aceton/hexane mixture using an ASE® 200 (Accelerated Solvent Extractor, Dionex, Sunnyvale, CA, USA). PAHs from supernatants were extracted with dichloromethane. Analysis of the PAH content in the extracts was performed according to a standardized method (Method EPA8270, Environmental Protection Agency, USA) by gas chromatography coupled with mass spectrometry (GC–MS). The MS detector used was a quadrupole mass spectrometer (Trace-MS; Fisons/Thermoquest, Belgium). The detection limit for the different PAH components was 0.2 μg $L^{-1}$. The quantification limit was 0.4 μg $L^{-1}$. The extraction efficiency of the sample preparation step prior to PAH analysis was between 80 and 110%, as determined with the reference soil CRM535.

**LC-MS analysis.** LC-MS analysis of the samples for hydroxy-PAHs was performed according to Van de Wiele et al. (2004). The identity of hydroxy-PAH metabolites in the samples was confirmed by using synthetic standards of these metabolites and compare the HPLC profiles from the colon digests with those from the standards. Briefly, all samples for LC-MS analysis were subjected to solid phase extraction using PrepSep$^{TM}$ C18 columns (250 mg) (Fisher Scientific, Edmonton, Canada). Sample volumes of 5ml were loaded on the columns and washed with 10 mL of Milli-Q ® water and subsequently, the target analytes were eluted with 10 mL of methanol. One mL aliquots were subsampled and stored in amber

vials at 4 °C prior to LC-MS analysis. HPLC analysis was performed using a Waters 2695 (Milford, MA, USA) separation module. The selected column was a 2.1 mm × 100 mm, 3.5 μm particle size, Waters XTerra MS C18 column (Milford, MA, USA) which was kept at a constant temperature of 26 °C. The binary eluent system consisted of methanol:water 90:10 v/v (eluent A) and methanol:water 10:90 v/v (eluent B). Mass spectrometry analysis was performed with a Quattro Ultima Mass spectrometer (Micromass Technologies, Manchester, UK) that was equipped with an electrospray interface operating in the negative ion mode. Instrumental control and data acquisition was performed with MassLynx software version 3.5. The electrospray ionization source was operated at 90°C, desolvation temperature 200 °C, cone voltage 61V, and a capillary voltage of 2.74 kV. Nitrogen gas served as the cone gas (flow rate of 159 L $h^{-1}$), desolvation gas (490 l $h^{-1}$) and nebulizer gas (set to maximum). The detector multiplier voltage was set to 650V. Selected ion monitoring was employed for quantitative analysis monitoring the $(M-H)^-$ of m/z for the PAH hydroxylates.

**Results and discussion.**

Due to their moderate to high degree of aromaticity, we expected pure solutions of naphthalene, phenanthrene, pyrene and benzo(a)pyrene to test positive in the Ah bioassay. Naphthalene, 200 nM, displayed 0.4 % benzo(a)pyrene equivalence whereas 200 nM phenanthrene and 200 nM pyrene displayed 15.1 % and 48.2 % benzo(a)pyrene equivalence. PAH compounds are not estrogenic and up to 16 µM of the four pure PAHs indeed did not induce an estrogenic response in the estrogen bioassay. Similarly, separate stomach and small intestine digests of the four PAHs did not show a significant estrogen response (Figure 2). In contrast, PAHs from colon digests became estrogenic. Conversion of the % EE2 equivalence values, depicted in Figure 2, to equivalent EE2 concentrations resulted for colon digests of 62.5 nM pyrene, into 2.70 nM EE2 equivalence, for phenanthrene, 2.14 nM EE2 equivalence, for benzo(a)pyrene, 1.48 nM EE2 equivalence and for naphthalene 0.31 nM EE2 equivalence. This PAH bioactivation was only evident in the colon digestion. This shows the selectivity of the colon digestion towards an increase in estrogenicity whereas no increased aryl hydrocarbon response was detected, compared to stomach or small intestine digests. To make sure that the observed effects were not coming from the matrix background of the colon interacting with PAHs, we incubated PAHs in a heat inactivated colon suspension. The removal of microbial activity markedly reduced the increase in estrogenic activity (Figure 2). This finding indicates that the risk for PAH bioactivation along the gastrointestinal tract is not exclusively associated with human biotransformation enzymes from the enterocytes in the small intestine epithelium and colonocytes in the large intestine epithelium (Autrup et al. 1978; De Kok and van Maanen, 2000; Doherty and Charman 2002), but that colon microbiota can also bioactivate PAHs.

We then evaluated the significance of this process using lower, more realistic concentrations obtained from a former urban playground soil, contaminated with 49±1.5 mg PAH $kg^{-1}$ soil DW by years of atmospheric deposition. *Pica* afflicted children form the largest risk group for soil ingestion due to their unusual hand-mouth behaviour and small body weight. Hence, we simulated the GI tract of a *pica* child, hypothetically ingesting 5 g soil $d^{-1}$. GC-MS analysis previously showed that the released PAH fraction from the soil matrix was highest in the stomach digest (18±5.3 µg $L^{-1}$), followed by the small intestine digest (3±1.1 µg $L^{-1}$) and the colon digest (2±0.3 µg $L^{-1}$) (Van de Wiele et al. 2004). This corresponded to a maximal Ah bioassay response for the stomach digest of 41±2.9% benzo(a)pyrene equivalence, the small intestine 27±1.4% and the colon 22±2.6% (Figure 3). Based on the role of the human Ah receptor in the toxicity of specific aromatic hydrocarbons, these findings would normally indicate that the colon digest represents the lowest risk for PAH bioactivation. Surprisingly, the trend in estrogenic activity was the inverse of observed PAH release or Ah bioassay response. Similar to the estrogen bioassay results on pure PAHs, there was negligable induction of estrogenic activity in the stomach (0.6%) and small intestine (2.0%) digestion (Figure 4). However, an average value of 20.1±0.84% EE2 equivalence was observed in a colon digestion of the contaminated soil (Figure 4). We infer that the PAH bioactivation potency from colon microbiota also occurs at lower and relevant concentrations for human exposure and that the presence of soil does not eliminate this potency.

Soil organic matter or nutritional fibres are known to lower the fraction of a contaminant that can be absorbed by the intestine (O'Neill et al. 1991; Oomen et al. 2000; van Schooten et al. 1990). This would theoretically lower the risk from ingested contaminants since bioactivation by human biotransformation enzymes will be reduced due to a lower bioavailability. To test this hypothesis, we compared the estrogenicity from colon incubated

PAHs in the presence and absence of soil by calculating the bioactivation potency of the digests as estrogenicity / aromaticity. We divided – at equimolar concentrations – the % EE2 equivalence of the different digests by their respective % benzo(a)pyrene equivalence. At equimolar concentrations of 8.03 nmol PAH $L^{-1}$, this ratio was for naphthalene, 0.93, for phenanthrene, 2.16, for pyrene, 0.98 and for benzo(a)pyrene, 0.12, whereas the colon digest of the PAH contaminated soil gave a ratio of 0.88, the same order of magnitude as the ratios for pure PAH compounds and one order of magnitude higher than the ratios for the stomach soil digest, 0.016, or small intestine soil digest, 0.077. These findings provide further evidence that the presence of a soil matrix does not eliminate the PAH bioactivation by colon microbiota and that the estrogenic potency of soil derived PAHs does not significantly decrease if compared to pure PAHs.

PAH metabolites that typically have estrogenic properties, are hydroxylated derivatives, due to their structural similarity to natural estrogens (Hirose et al. 2001; Fertuck et al. 2001). Hence, in a next step of the research, we screened with LC-MS for the presence of hydroxy-PAHs by analyzing the respective colon digests of 20 μmol $L^{-1}$ pure PAH compounds. The identity of PAH metabolites was confirmed by comparing the HPLC profiles and MS spectra of the colon digests with those from chromatographic synthetic standards of several hydroxy-PAHs. The developed protocol had reasonably low detection limits for 1-hydroxy napthalene, 9-hydroxy phenanthrene, 1-hydroxy pyrene and 7-hydroxy benzo(a)pyrene (Table 1) (Van de Wiele et al. 2004). No hydroxy-PAHs were detected upon stomach or small intestine incubations. From all colon digests, only the pyrene digest tested positive for a hydroxylated PAH metabolite with 1-OH pyrene at a concentration of 2.5 μg $L^{-1}$ (Table 1). Glucuronidated or sulfated PAH conjugates are also typical biotransformation products from eukaryotic organisms (Cajthaml et al. 2002). Since the concentration of fungi

and yeasts in the colon suspension amounted to 4.3±0.6 log CFU $mL^{-1}$, we tested whether PAH conjugates were present in colon digests of pure PAH compounds. Glucuronidase and arylsulfatase typically cleave off glucuronic acid or sulfate groups from conjugated PAHs, regenerating the hydroxylated PAH metabolites (Cajthaml et al. 2002). After incubating the extracts of the colon digests in the presence of glucuronidase (100 U $mL^{-1}$) and arylsulfatase (60 U $mL^{-1}$) for 6 hours at 37°C, we found higher concentrations of 1-OH pyrene (4.4 µg $L^{-1}$) and a new metabolite, 7-OH benzo(a)pyrene (1.9 µg $L^{-1}$). No hydroxylated PAHs were retrieved from inactivated colon samples. Although other PAH hydroxylates may have formed than those tested during LC-MS analysis, these analytical data show that PAH bioactivation by colon microbiota may result from hydroxylated PAH metabolites.

The formation of hydroxylated PAH metabolites and especially the increased estrogenicity by human colon microbiota bring up two questions. Are the observed transformations plausible for the *in vivo* human gastrointestinal tract and to what extent can bioactive PAH metabolites contribute to the total risk from oral PAH exposure? To answer the first question, literature shows that resident gut microbiota may influence xenobiotic metabolism from the intestinal epithelium (Hooper et al. 2001). Additionally, microbial glucuronidase activity in the intestine sometimes cleaves off glucuronic acid groups from excreted human conjugated metabolites, thus regenerating the more bioactive hydroxylated intermediates (Aura et al. 2002). These reports describe indirect effects of intestinal microbiota towards xenobiotic metabolism. However, our findings indicate a direct metabolism effect of human colon microbiota towards PAH parent compounds, since the in vitro approach used in the current study eliminated possible interferences by intestinal epithelium enzymes. The observed biotransformation and bioactivation reactions originate from a microbial community which resembles that of the *in vivo* intestinal lumen both in

composition and in metabolic activity. Rather than containing the less active microbiota from fecal matter, the microbial community from the used *in vitro* method is more representative of the different parts of the human colon (Molly et al. 1993).

As suggested by the LC-MS results, the colon microbiota formed hydroxylated PAH metabolites, which may seem unlikely since this oxidative step would occur in an anaerobic environment as shown by redox potential values from the colon suspension that varied between -180 mV and -230 mV. The latter values are well within the range of -145 mV to -250 mV reported for the colon *in vivo* (Bowler et al. 2001; Chourasia and Jain 2003). Yet, oxidative reactions by intestinal bacteria from humans, mice and rats have been described for the conversion of 2-amino-3-methylimidazo[4,5-f] quinoline (IQ) to its reportedly mutagenic 7-keto derivative (7-OHIQ) (Rumney et al. 1993). Additionally, *Enterococcus faecalis* even performs aromatic hydroxylation reactions in the intestine *in vivo* (Huycke and Moore 2002). It is therefore not unlikely that intestinal microbiota may hydroxylate PAHs, also given the fact that anaerobic PAH hydroxylation has been reported by microorganisms, albeit in sediments (Karthikeyan and Bhandari 2001). These studies on oxidative reactions by intestinal microbiota and anaerobic PAH biotransformations may thus support our findings, which need further study to identify which microorganisms bioactivate PAHs.

To answer the second question concerning the contribution of the observed effects to the total risk from PAH ingestion, further research is warranted. Yet, the microbial PAH bioactivation to estrogenic metabolites may constitute an increased health risk when the human body is orally exposed to contaminated soils. The human colon epithelium is 20% more permeable to 17β-estradiol than the human small intestine epithelium (van der Bijl and van Eyck 2003) and also has a higher permeability to hydrophobic compounds in general (Ungell et al. 1998). PAH metabolites with structures resembling steroidal hormones may

thus exhibit weak estrogenic or antiestrogenic activity *in vivo* (Ariese et al. 2001). Since PAHs that reach the colon will be biotransformed by colon microbiota, we conclude that, in the *in vivo* situation, the colonic epithelium – which has estrogen receptors – may be subjected to hazardous effects from microbial PAH metabolites. The equivalent EE2 response of 20 % for the colon incubated environmental sample (Figure 4) indicates that the observed activation of the human estrogen receptor is significant. Still, it must be kept in mind that a positive response in ER-reporter gene assays such as that from the present study, does not necessarily predict endogenous transcription (Gozgit et al., 2004). Gozgit et al. (2004) noted that several PAHs induced activity in ER-reporter gene assays but that these PAHs did not upregulate estrogen-responsive genes. The authors conclude that the ER-reporter gene assays may detect concentrations of toxicants that are not physiologically active. In light of these recent findings, the estrogenic response from microbial PAH bioactivation in this study needs careful interpretation. However, the finding of 1-OH pyrene and 7-OH benzo(a)pyrene as metabolites from human colon microbiota is something that is not anticipated from current scientific knowledge or risk assessment studies. Comparison of our findings from active to those from inactivated colon microbiota shows us that the microbial bioactivation potency is a factor 12 higher than would be currently expected in risk calculations. Moreover, the time during which bioactive hydroxy-PAHs could react with colonocytes is also considerably longer (up to 72 hours) than the residence time in human enterocytes or hepatocytes (6 hours at maximum). Additionally, if taken up by colonocytes, the hydroxy-PAHs are typical metabolites that are more easily metabolized by human biotransformation enzymes to for example potent carcinogens such as b(a)p-r7,t8-dihydrodiol-t9,10-epoxide (Kim et al., 1998). Clearly, these literature reports on human PAH metabolism and our findings of PAH bioactivation by colon microbiota indicate the importance of conducting future work in which

the relative importance of the human bioactivation processes versus the microbial bioactivation processes should be compared.

**Conclusion.**

In summary, our results reveal that human colon microbiota can directly bioactivate PAHs, a potency that has not been reported before. As indicated by the analysis of a PAH contaminated environmental sample, we also show that the presence of soil does not eliminate this microbial bioactivation potency. We therefore conclude that risk calculations that are solely based on human biotransformation enzymes, may underestimate the risk from ingested aromatic contaminants because it does not consider the bioactivation processes described here.

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**Table 1.** Limits of detection of hydroxy-PAHs as obtained with LC-MS analysis, concentrations of the hydroxy-PAHs in the colon digest, and concentrations of the hydrxoy PAHs in the deconjugated colon digest

| | **LOD ($\mu g\ L^{-1}$)** | **Colon digest ($\mu g\ L^{-1}$)** | **Colon digest deconjugated ($\mu g\ L^{-1}$)** |
|---|---|---|---|
| 1-OH naphthalene | **7.33** | **-** | **-** |
| 9-OH phenanthrene | **0.47** | **-** | **-** |
| 1-OH pyrene | **0.24** | **2.5** | **4.4** |
| 7-OH benzo(a)pyrene | **0.95** | **-** | **1.9** |

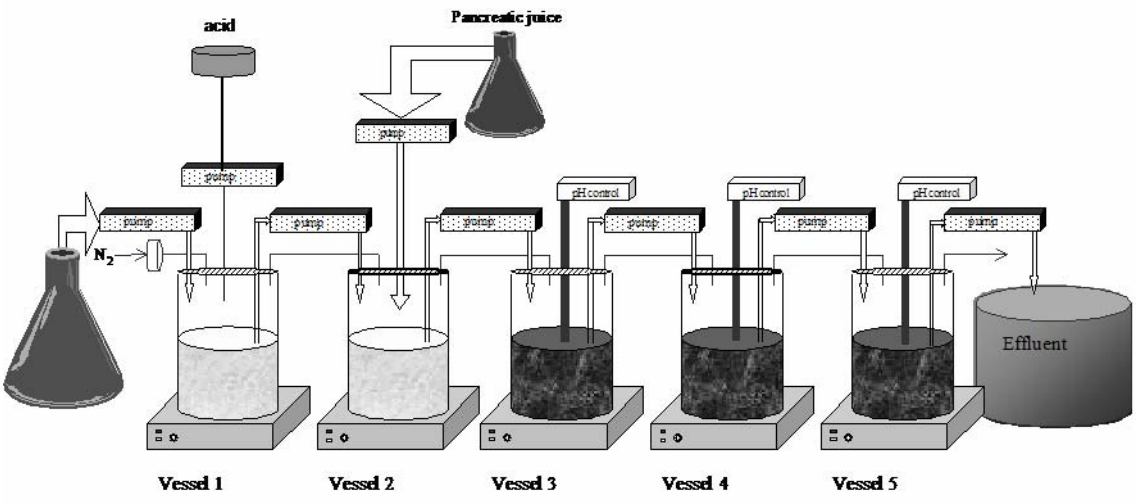


**Figure 1.** Schematic representation of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). Vessels 1 to 5 respectively simulate conditions from the stomach, small intestine, colon ascendens, colon transversum and colon descendens.

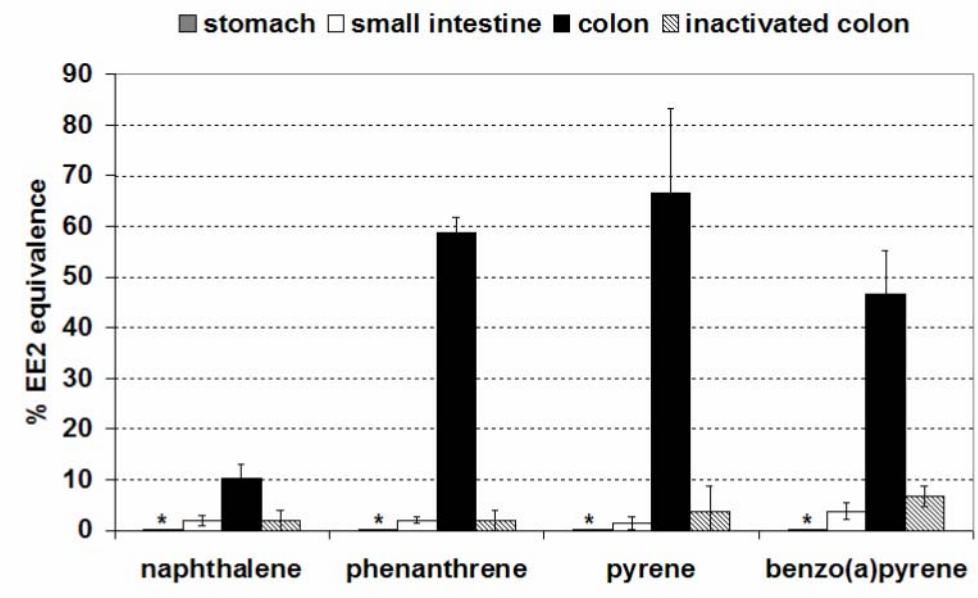


**Figure 2.** Estrogen response of naphthalene, phenanthrene, pyrene and benzo(a)pyrene incubated at 62.5 nmol/L in stomach, small intestine, colon digests and digests with inactivated colon microbiota. Values are means of 4 replicates. Error bars represent standard deviation values. * none of the stomach digestions gave a significant response in the estrogen bioassay.

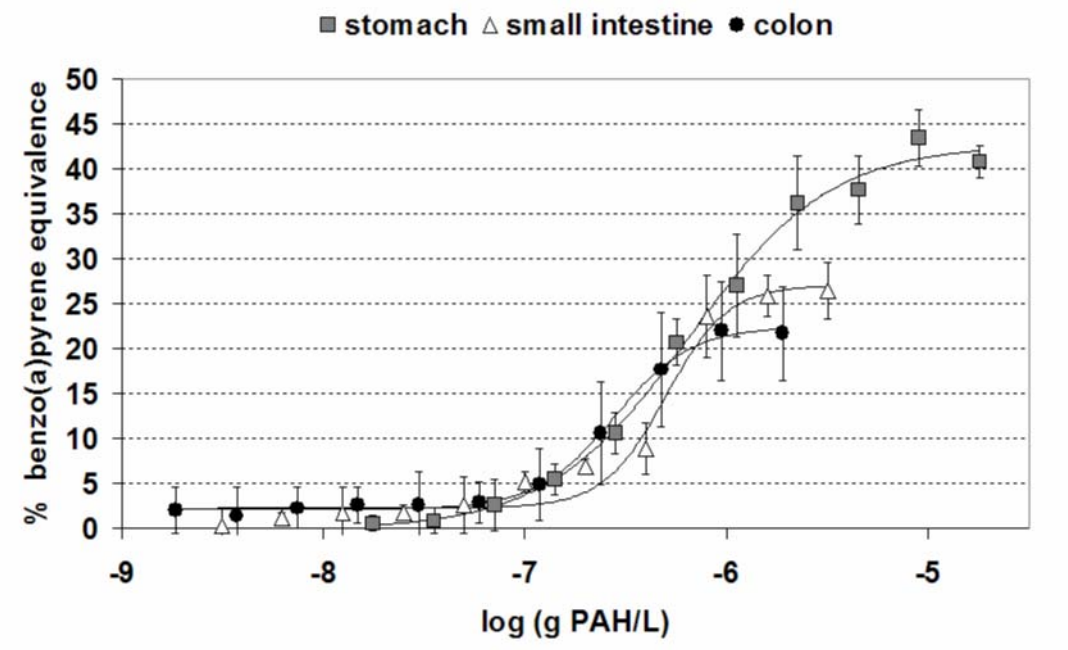


**Figure 3.** Dose-response curve of stomach, small intestine and colon digests of a PAH contaminated playground soil in the Ah receptor yeast bioassay, expressed as percentage benzo(a)pyrene equivalence in function of released PAH concentration in the respective digests. Values are averages of 4 replicates. Error bars represent standard deviation and may disappear in the datapoint if too small.

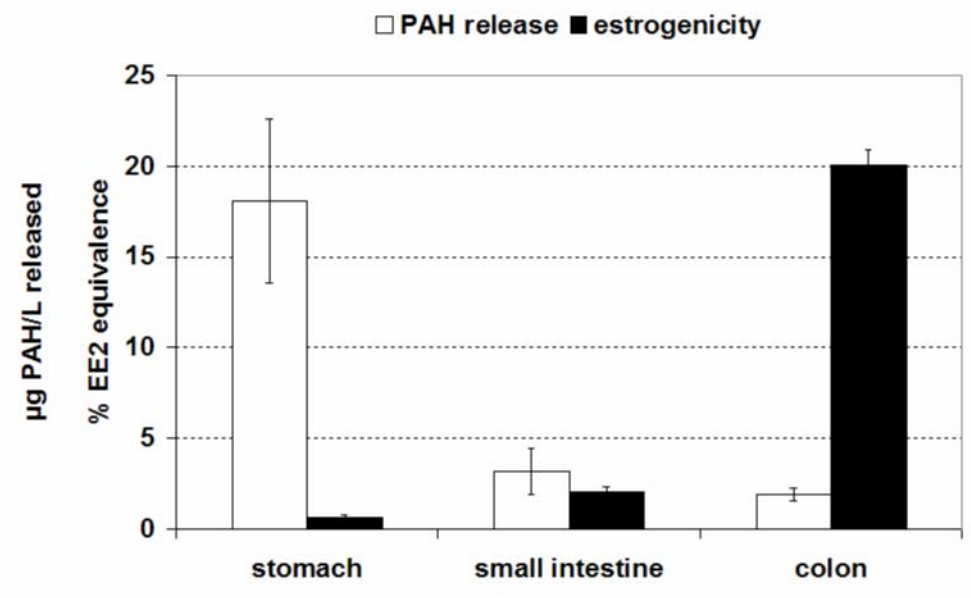


**Figure 4.** Released concentrations of PAHs and estrogen response in stomach, small intestine and large intestine digests incubated with PAH contaminated soil samples. Error bars represent standard deviation values of 4 replicates.